

# Nuclease

## Week 16

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

### Summary

<b>1</b>	<b>Inoculation of pSB1C3-T7-Nuc glycerol stock sample in liquid media</b>	<b>2</b>
<b>2</b>	<b>Kirby-Bauer test on expressed nuclease</b>	<b>3</b>
<b>3</b>	<b>Biofilm assay on pSCB1C3-T7-Nuc</b>	<b>5</b>
<b>4</b>	<b>Biofilm assay on pSCB1C3-T7-Nuc - second attempt</b>	<b>9</b>

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# **1 Inoculation of pSB1C3-T7-Nuc glycerol stock sample in liquid media**

## **Responsible**

Aman Mebrahtu

## **Results and Conclusions**

Successful.

## 2 Kirby-Bauer test on expressed nuclease

### Responsible

Reskandi Rudjito

### Protocols used

Kirby-Bauer test

### Modifications and comments to protocols

Protein/antibiotic samples:	Kanamycin	35 mg/ml (positive control)
	dH <sub>2</sub> O	(negative control)
	Nuc P	0.5 mM ITPG
	Nuc P	1.0 mM ITPG
Incubation time		16 h
Volume of TOB1 cells on McConkey plates		200 $\mu$ l

### Experimental Set Up

Two plates were prepared with Nuc pellet (P). Kanamycin was used as a positive control and dH<sub>2</sub>O as a negative control on both plates. Whatman papers were impregnated with 200  $\mu$ l of sample, positive control, and negative control.

### Results and Conclusions

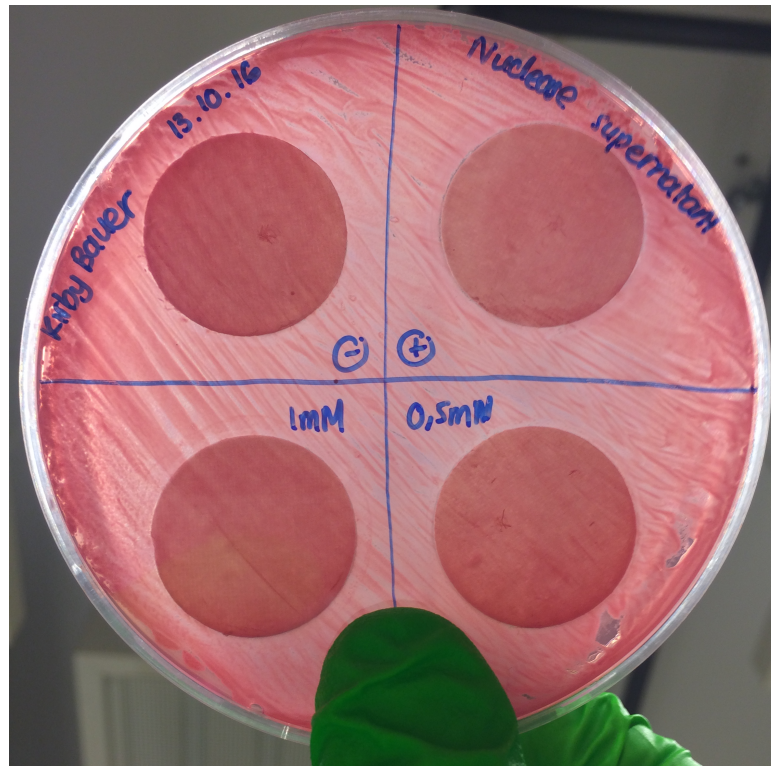


Figure 1: Result of the incubation of TOB1 cells on MacConkey agar with impregnated Whatman paper discs.

## Discussion and Troubleshooting

The results from this attempt were inconclusive since it was difficult to determine whether the growth of TOB1 around the impregnated Whatman papers was actually impeded. More clear results of the activity of nuclease were obtained from the biofilm assay of the same cell lysate samples.



### 3 Biofilm assay on pSCB1C3-T7-Nuc

#### Responsible

Shuangjia Xue, Jenny Waspe, and Alik Mitropoulou

#### Protocols used

Biofilm Assay

#### Experimental Set Up

Biofilm dispersal by Nuc was demonstrated using crystal violet assays; one with *P. aeruginosa* as the biofilm-producer and the other with *S. aureus*. Induced BL21(DE3) cell lysate samples (both debris pellet resuspension and supernatant) are used for the experiment. The biofilm is treated with 1  $\mu$ l, 5  $\mu$ l, 10  $\mu$ l, or 20  $\mu$ l of Nuc sample. Differences between the negative control and Nuc are evaluated using the students t-test. A p-value  $\leq 0.05$  is considered statistically significant. The samples from supernatants are labelled "Nu S". The samples from the pellets were contaminated and are not shown in the results.

A crystal assay is conducted to evaluate how Nuc inhibits biofilm growth by *P. aeruginosa* and *S. aureus*, respectively. Induced BL21(DE3) cell lysate samples (both debris pellet resuspension and supernatant) are used for the experiment.

*S. aureus* is cultured in BHI media and *P. aeruginosa* PAO in LB media. After an overnight incubation, the cell density was measured at OD 600. 200  $\mu$ l of the liquid culture is added into each well and treated with Nuc of four different volumes; 1, 5, 10 and 20  $\mu$ l. The biofilm is allowed to develop for 48 hours with subsequent staining. OD is then measured again at 600 nm. Cell lysates of BL21(DE3) cells are used as negative control. Differences between the negative control and Nuc is evaluated using the students t-test. A p-value  $\leq 0.05$  is considered statistically significant. The samples from supernatants and pellet are labelled "Nuc S" and "Nuc P", respectively.

## Results and Conclusions

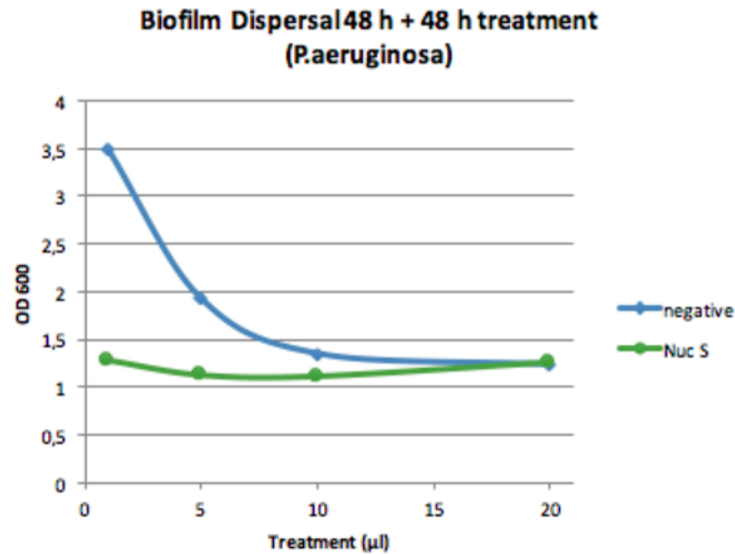


Figure 2: OD measured for different treatments of Nuc using *P. aeruginosa* as biofilm-producer.

The graph illustrates how different volumes of Nuc dispersed biofilm formed by *P. aeruginosa*. There is a significant difference ( $p=0.023$ ) between the negative control and 1 μl of Nuc treatment. Notably, OD decreases as the amount of negative control increases. This may be due to contamination or elution or dispersal of biofilm because of the increased amount of fluid *per se* in the treatment.

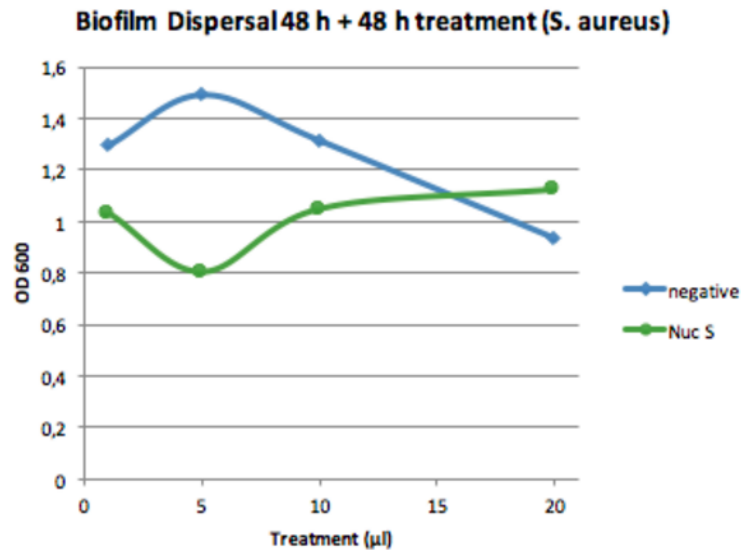


Figure 3: OD measured for different treatments of Nuc using *S. aureus* as biofilm-producer.

The graph illustrates how different volumes of Nuc dispersed biofilm formed by *S. aureus*. There is a significant difference between 5 μl of negative control and 5 μl of Nuc S.

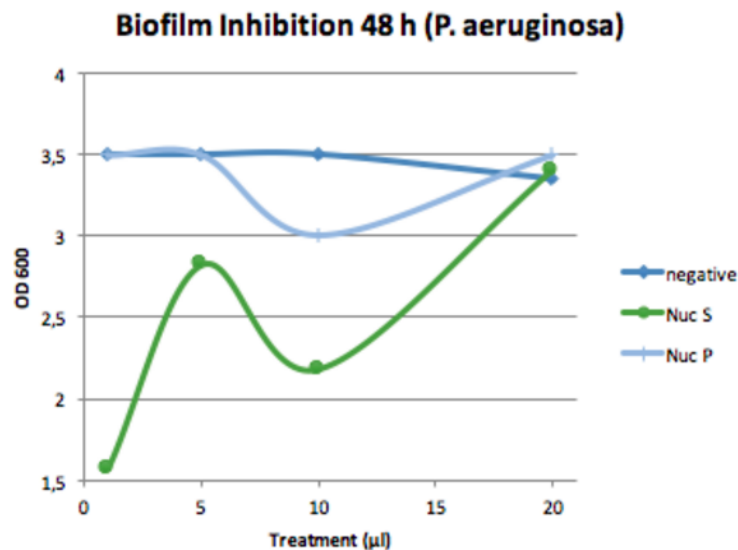


Figure 4: OD measured for different treatments of Nuc using *P. aeruginosa* as biofilm-producer.

The graph illustrates how the combination of supernatant and resuspended pellet of Nuc cell lysate inhibits the formation of biofilm by *P. aeruginosa*. As the treatment increases, the OD tends to increase as well. There is a significant difference ( $p=0.02$ ) between the negative control and Nuc S for the 1 μl treatment.

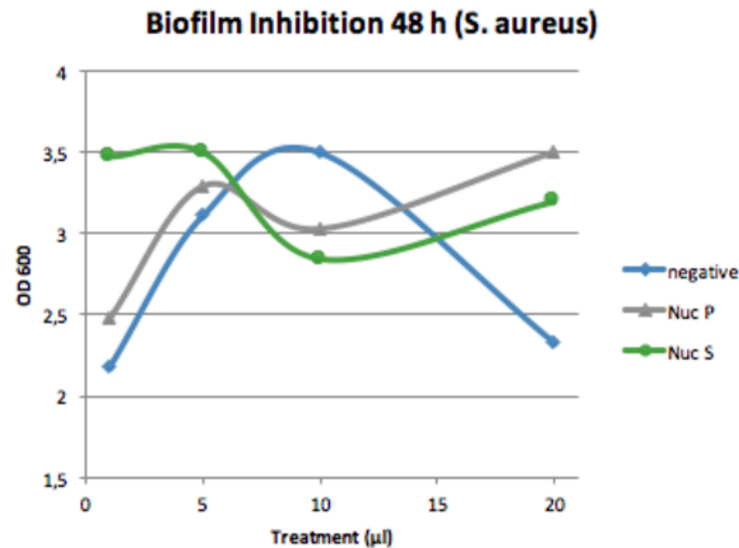


Figure 5: OD measured for different treatments of Nuc using *S. aureus* as biofilm-producer.

The graph illustrates how the combination of supernatant and resuspended pellet of Nuc cell lysate inhibits the formation of biofilm by *S. aureus*. The negative control seems inconsistent; it may be that it is dose dependent in *S. aureus* or that it has been contaminated. This experiment showed a significant difference between 1  $\mu$ l of negative control and Nuc S, as well as between 20  $\mu$ l of negative control and Nuc P. However, these results may not be reliable.

## 4 Biofilm assay on pSCB1C3-T7-Nuc - second attempt

### Responsible

Shuangjia Xue, Jenny Waspe, and Alik Mitropoulou

### Protocols used

Biofilm Assay

## Experimental Set Up

The second attempt of dispersing biofilm was similar to the first. However, this time a combination of pellet and supernatant was used as treatment with the ratio 1:1, e.g. for 5  $\mu$ l treatment, 2.5  $\mu$ l of each was used. For this attempt the biofilm was treated with 5  $\mu$ l, 20  $\mu$ l and 50  $\mu$ l of Nuc. The samples were labelled "Nuc" and the negative control was labelled "Neg". Only one volume of negative control was used.

The second attempt of inhibiting biofilm growth was similar to the first. However, this time a combination of pellet and supernatant was used as treatment with the ratio 1:1, e.g. for 5  $\mu$ l treatment, 2.5  $\mu$ l of each was used. For this attempt the biofilm was treated with 5  $\mu$ l, 20  $\mu$ l and 50  $\mu$ l of Nuc. The samples were labelled "Nuc" and the negative control was labelled "Neg". Only one volume of negative control was used.

## Results and Conclusions

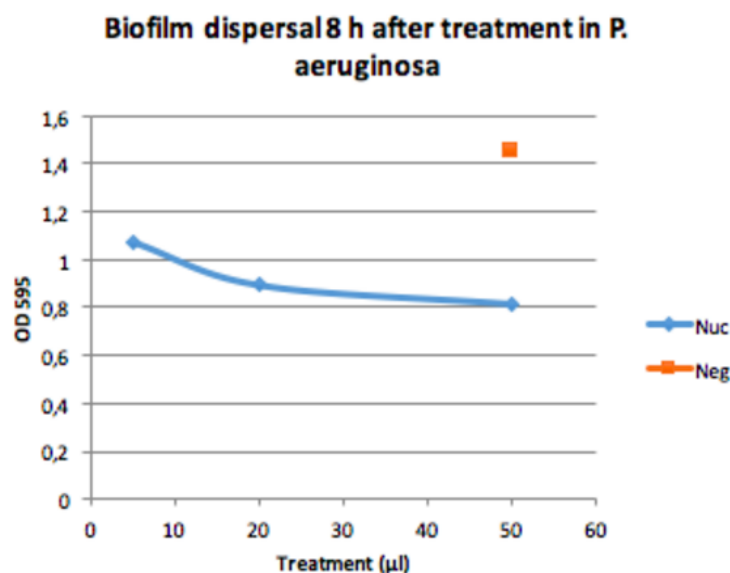


Figure 6: OD measured for different treatments of Nuc using *P. aeruginosa* as biofilm-producer.

The figure illustrates how Nuc dispersed biofilm produced by *P. aeruginosa*. There is a significant difference between the negative control and each of the three volumes of Nuc. As the volume of Nuc increases, OD tends to decrease. This implicates that Nuc significantly dispersed biofilm compared to the negative control.

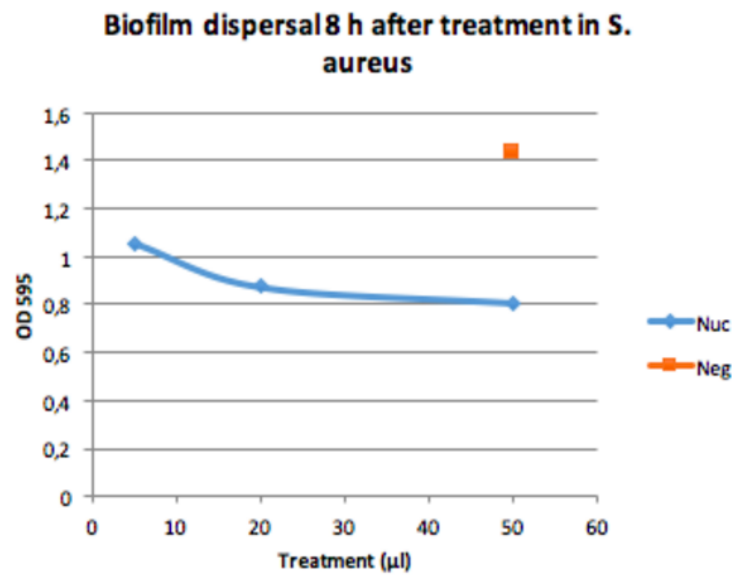


Figure 7: OD measured for different treatments of Nuc using *S. aureus* as biofilm-producer.

The figure illustrates how Nuc dispersed biofilm produced by *S. aureus*. There is a significant difference between the negative control and each of the three volumes of Nuc. This implicates that Nuc significantly dispersed biofilm compared to the negative control.

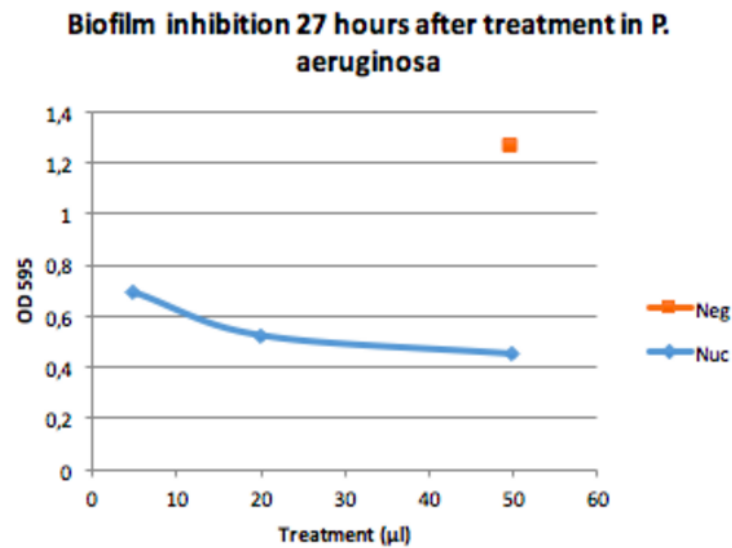


Figure 8: OD measured for different treatments of Nuc using *P. aeruginosa* as biofilm-producer.

The figure illustrates how Nuc (supernatant and pellet in a 1:1 ratio) inhibits biofilm formation by *P. aeruginosa*. These results show that OD decreases as the volume of treatment increases. There is a significant difference between the negative control and 20 µl and 50 µl of Nuc treatment, respectively.

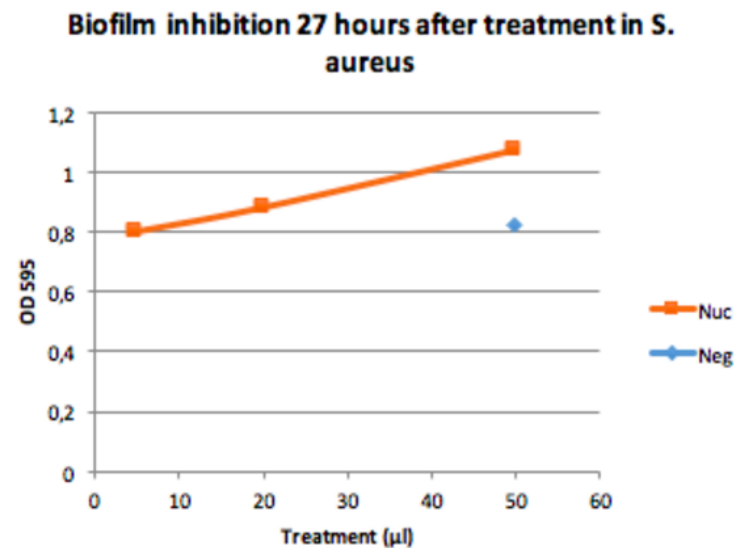


Figure 9: OD measured for different treatments of Nuc using *S. aureus* as biofilm-producer.

The figure illustrates how Nuc (supernatant and pellet in a 1:1 ratio) inhibits biofilm formation by *S. aureus*. The poor readout of the negative control implicates that the test is invalid.

However, OD of biofilm increases as volume of Nuc increases, and the negative control shows low values. There is a significant difference between the negative control and 50  $\mu$ l of Nuc.